Engineered Antibodies for the Treatment of Anthrax

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INFECTION AND IMMUNITY, Dec. 1997, p. 5171–5175 Vol. 65, No. 12
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Passive Protection by Polyclonal Antibodies against *Bacillus anthracis* Infection in Guinea Pigs

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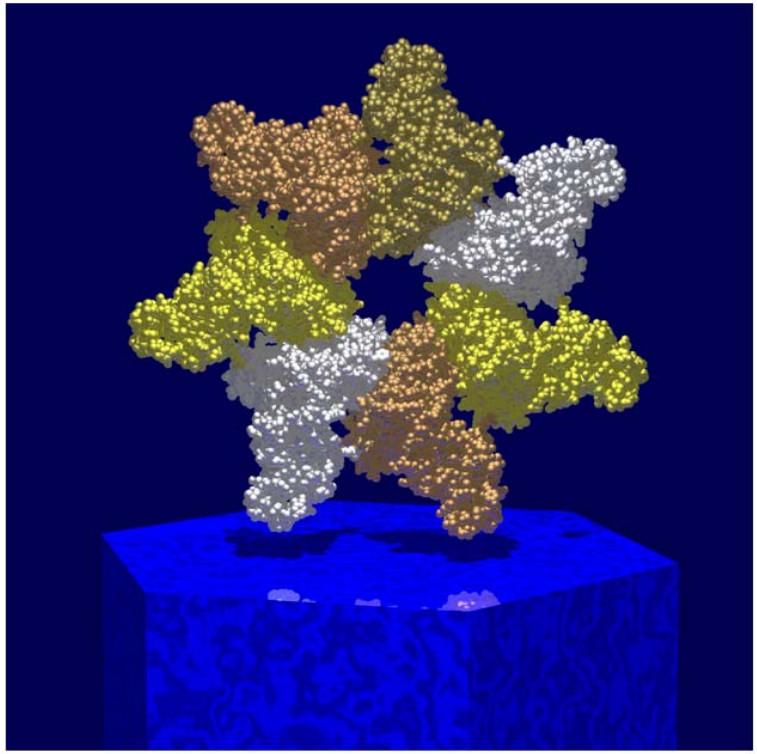
Received 17 June 1997/Returned for modification 9 July 1997/Accepted 24 September 1997

The protective effects of polyclonal antisera produced by injecting guinea pigs with protective antigen (PA), the chemical anthrax vaccine AVA, or Sterne spore vaccine, as well as those of toxin-neutralizing monoclonal antibodies (MAbs) produced against PA, lethal factor, and edema factor, were examined in animals infected with *Bacillus anthracis* spores. Only the anti-PA polyclonal serum significantly protected the guinea pigs from death, with 67% of infected animals surviving. Although none of the MAbs was protective, one PA MAb caused a significant delay in time to death. Our findings demonstrate that antibodies produced against only PA can provide passive protection against anthrax infection in guinea pigs.



Antibodies to the PA toxin could serve two roles:

1. As a prophylactic to prevent spore germination in analogy to the vaccine (passive immunization)
2. As a late stage antitoxin to serve as an antidote past the point at which antibiotic therapy is effective

First Generation Enhanced 14B7

Antibody Variant	k_{on} (*10^5 M^{-1} sec^{-1})	k_{off} (*10^{-4} sec^{-1})	K_d (nM)
14B7 scAb	**2.8 ± 0.3**	**30 ± 0.8**	**12**
1H scAb	**6.1 ± 0.9**	**1.6 ± 0.4**	**0.26**

Nat. Biotech., 2002, 20, 597-601

Lethal Toxin Challenge

Animal Model --> ***Rat***

Antidote ***--> Inject 4x and 1.5x Excess of Antibody 5 Minutes Prior to Toxin***

Challenge --> ***Venous Injection of Toxin*** ***(10X Lethal Dose)***

Lethal Toxin Challenge

Treatment	*K_d (nM)*	*TTD (min) **	*Survivors*
PBS	**-**	**82,87,92,97,99**	**0/5**
L97 scFv	**63**	**64,66,67,70,77**	**0/5**
14B7 scFv	**12**	**85,103,112,123,130**	**0/5**
A2E scFv	**4**	**171,242,271**	**2/5**
1H scFv	**0.25**	**212,238**	**3/5**
14B7 scAb	**12**	**102,115,140,172,292**	**0/5**
1H scAb	**0.25**	**--**	**5/5**
1H scAb, (1.5X concentration)	**0.25**	**152**	**4/5**

***Total time of experiment 5 hrs**

Jennifer Maynard

Nat. Biotech., 2002, 20, 597-601

Lethal Toxin Challenge

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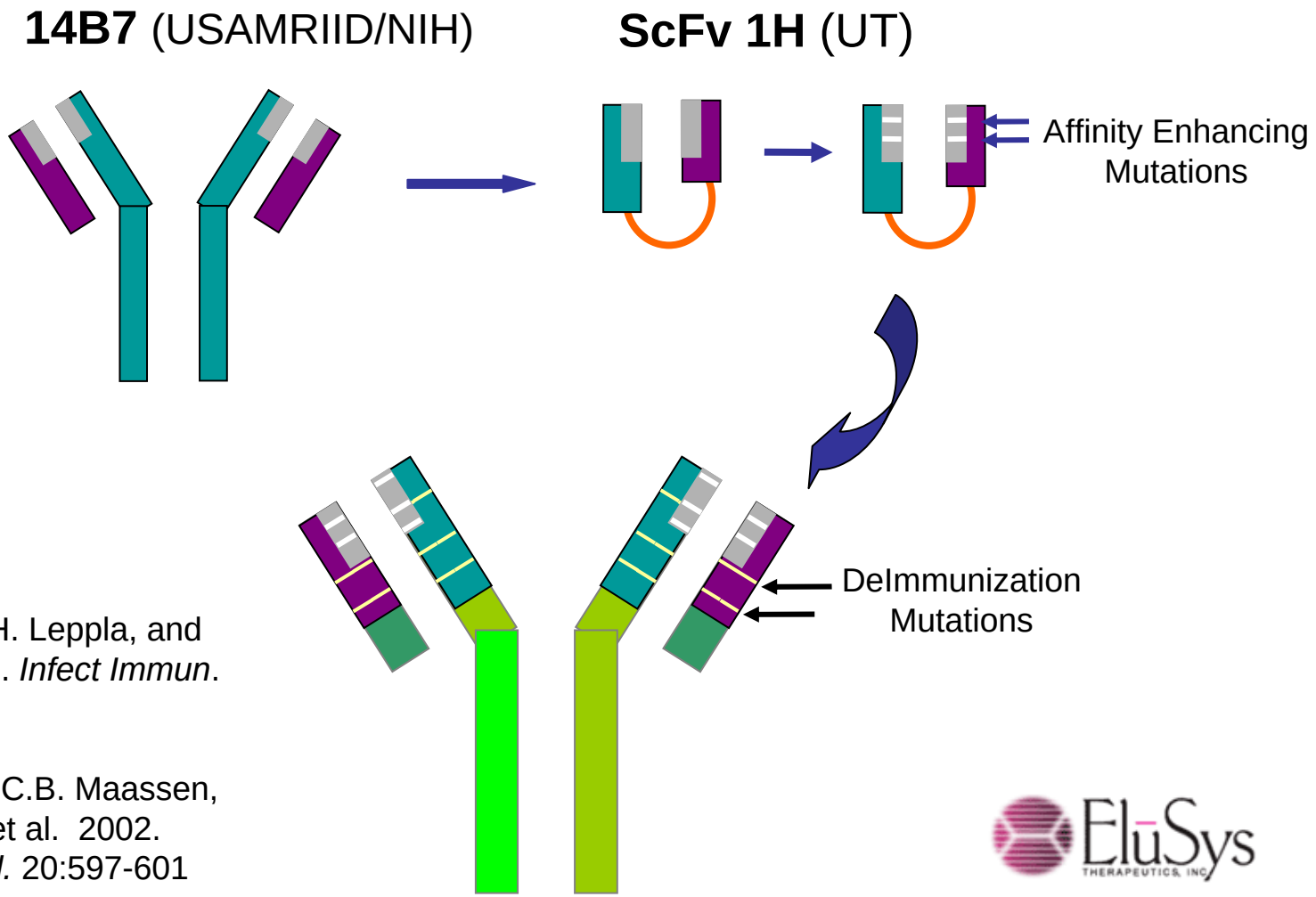
Nat. Biotech., 2002, 20, 597-601

Engineering of Anthim™-NIH Funded



Little S.F., S. H. Leppla, and E. Cora. 1988. *Infect Immun*. 56:1807-13.

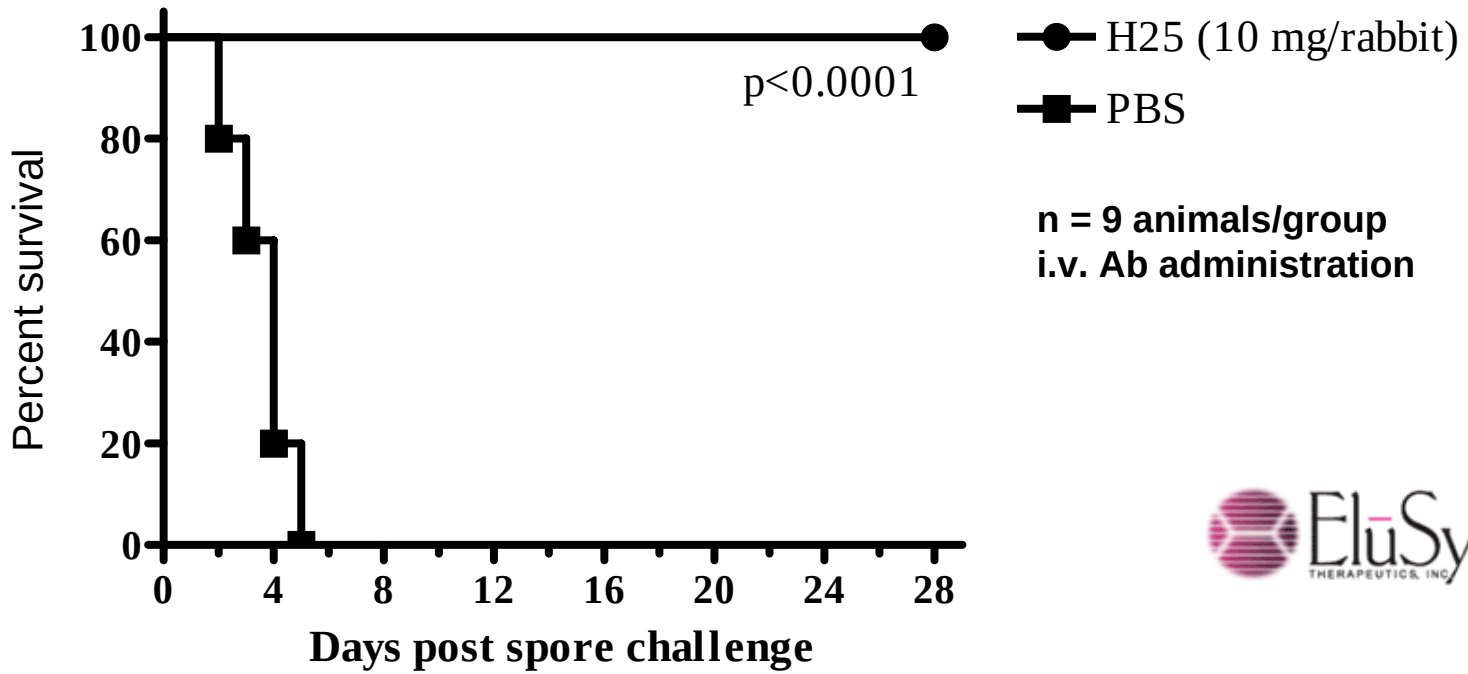
Maynard J.A., C.B. Maassen, S. H. Leppla, et al. 2002. *Nat Biotechnol.* 20:597-601

EluSys THERAPEUTICS, INC.

Spore Challenge

Can the antibody act as a prophylactic treatment?

Rabbit Model 100-300 LD_{50}'s Aerosol Challenge (Ames)



Leslie Casey, Nehal Mohamed, EluSys, Inc.

Infect. Immun. 2005, 73, 795-802

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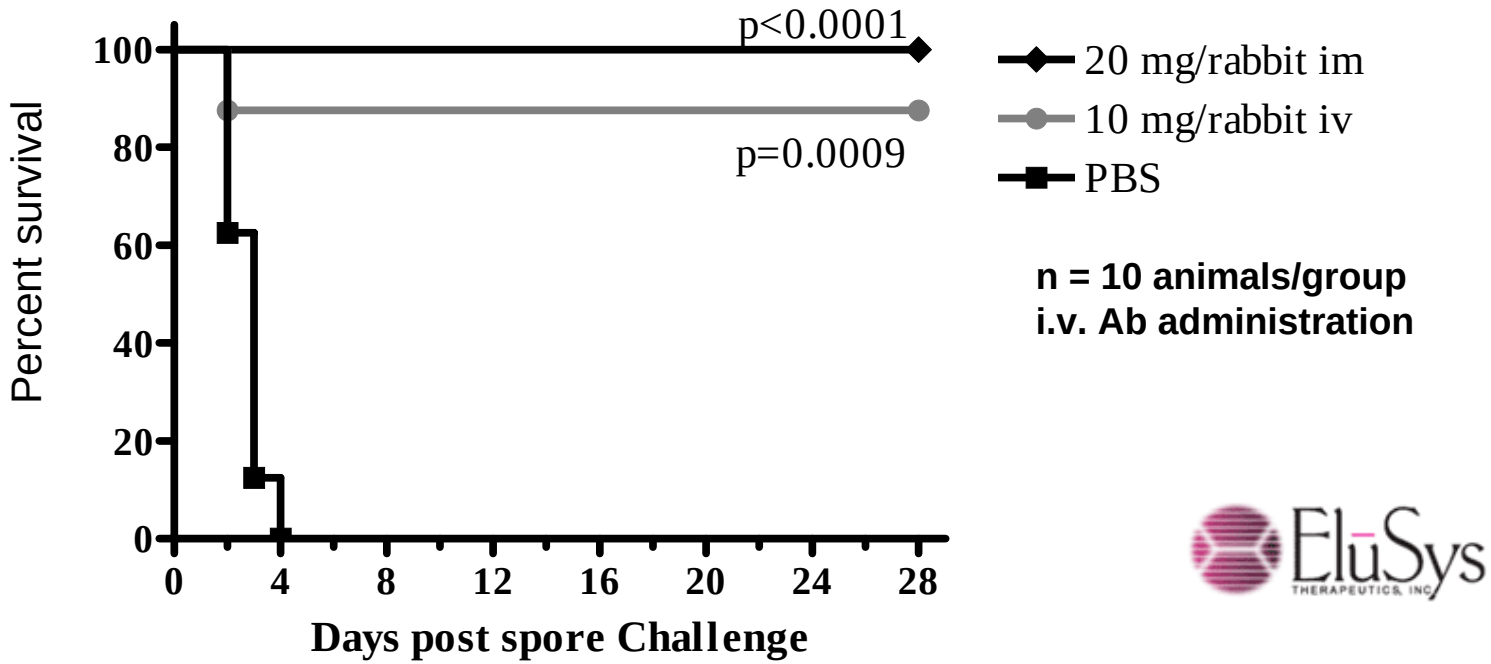


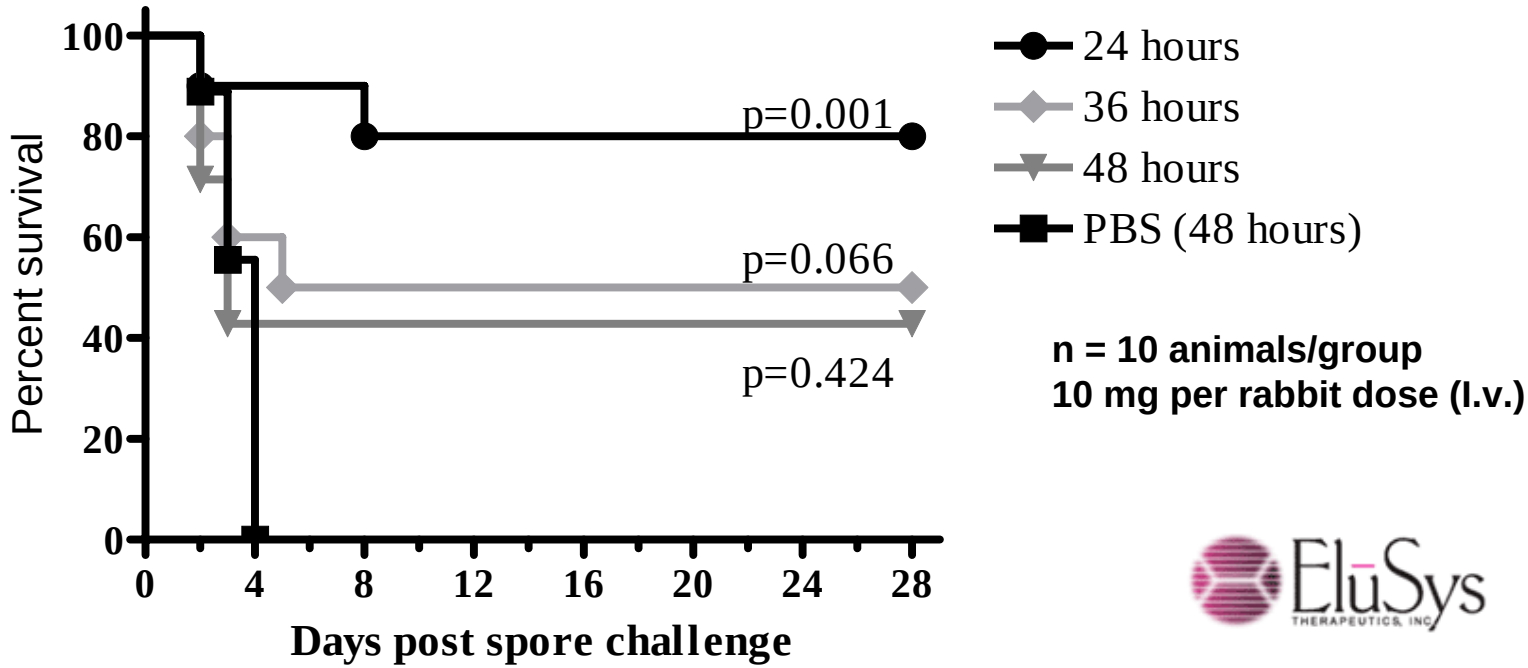
Leslie Casey, Nehal Mohamed, EluSys, Inc.

Infect. Immun. 2005, 73, 795-802

Spore Challenge

Can the antibody act as a post-exposure treatment?

Rabbit Model 100-300 LD_{50}'s Aerosol Challenge (Ames)



n = 10 animals/group
10 mg per rabbit dose (I.v.)

EluSys THERAPEUTICS, INC.

Leslie Casey, Nehal Mohamed, EluSys, Inc.

Infect. Immun. 2005, 73, 795-802

Anthim™ Treated Rabbits Are Free of Bacilli in Blood, Spleen

Animals positive for bacteria in blood								
Group	day1	day2	day7	day10	day14	day21	day28	At Death
Anthim™	0/9	0/9	0/9	0/9	0/9	0/9	0/9	--
PBS	0/5	3/5						4/5

Animals positive for bacteria in organs day 28			
Group/Organ	Lung	Lung-assoc. nodes	Spleen
Anthim™	2/9	1/9	0/9

Leslie Casey, Nehal Mohamed, EluSys, Inc.

Infect. Immun. 2005, 73, 795-802

Anthim™ Treated Mice Are Free of Bacilli in Spleen

Intratracheal challenge, 1xLD100 Sterne in DBA mice (CR Lyons, UNM)

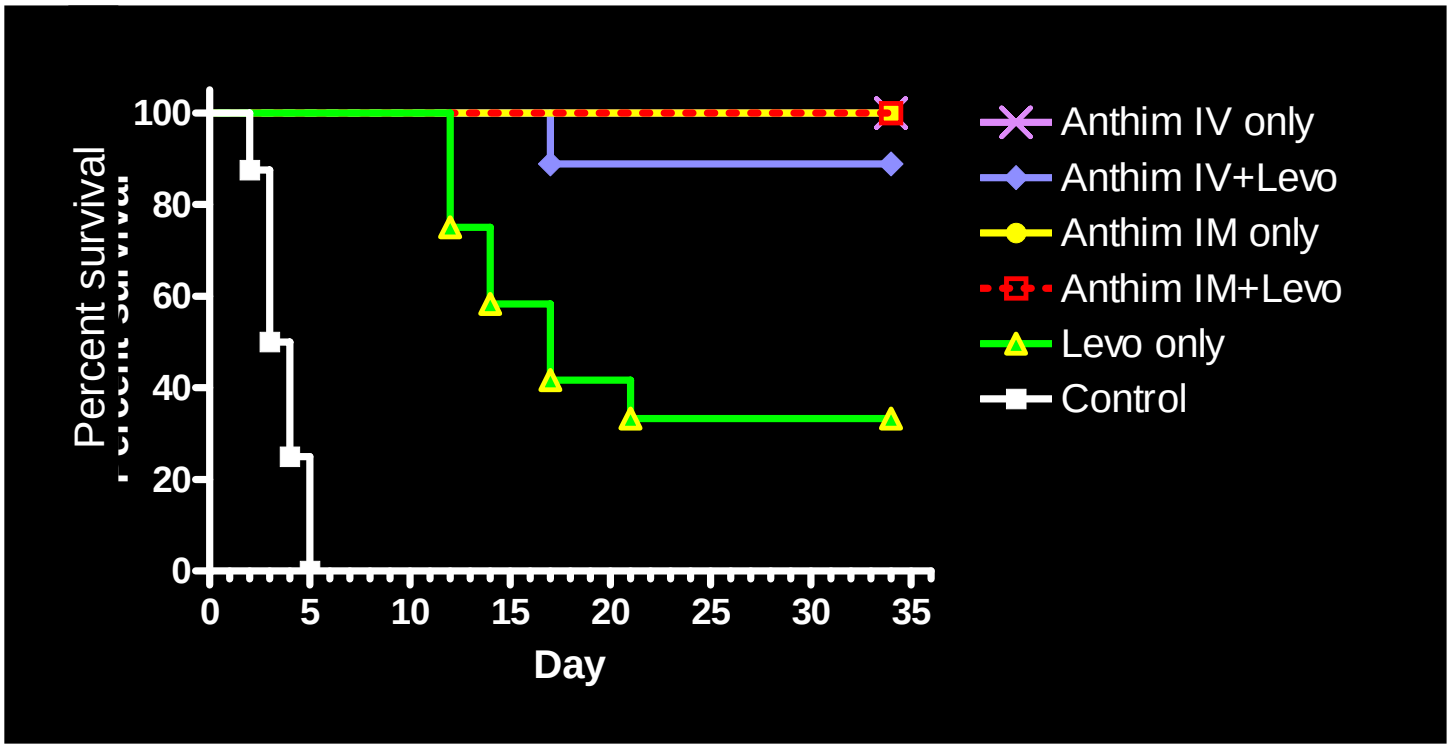
Mouse ID	CFU/ Lung (Day 15)	CFU/ Spleen (Day 15)
14B7, **1**	**19.6 X 10^4**	**bd***
14B7, **2**	**13.5 X 10^4**	**bd**
14B7, **3**	**10.8 X 10^4**	**0.04 X 10^4**
Anthim™, **1**	**1.7 X 10^4**	**bd**
Anthim™, **2**	**13.4 X 10^4**	**bd**
Anthim™, **3**	**10.5 X 10^4**	**bd**
Anthim™, **4**	**1.2 X 10^4**	**bd**
Anthim™, **5**	**1.1 X 10^4**	**bd**

Control mice all died. *bd = below detection

EluSys THERAPEUTICS, INC.

AR007: Rabbit Spore Challenge +/- Fluoroquinolone

Objective: To demonstrate that post-exposure administration of Anthim™ by the IV or IM route leads to increased survival above that of levofloxacin after spore challenge



ElūSys THERAPEUTICS, INC.

Clinical Evaluation of Anthim™

AH-101: Dose-Escalation Phase 1

- Safety, tolerability, PK of single IV dose of Anthim (ETI-204). Screen for interaction with ciprofloxacin (cipro)
  - Randomized, placebo-controlled
  - double blind

Part 1: Single dose Anthim infused IV over 90 min. at 3 dose levels

1. 19 mg; 6 Anthim, 2 PBO, M/F
2. 57 mg; 6 Anthim, 2 PBO, M/F
3. 114 mg; 6 Anthim, 2 PBO, M/F

Part 2: Single dose Anthim infused IV over 90 min. at highest dose level +/- 500 mg BID cipro for 14 days, 6 subjects/group

1. 114 mg; Anthim + cipro
2. Control; PBO + cipro

EluSys THERAPEUTICS, INC.

Clinical Evaluation of Anthim™

AH-101: Dose-Escalation Phase 1

- Safety, tolerability, PK of single IV dose of Anthim (ETI-204). Screen for interaction with ciprofloxacin (cipro)
  - Randomized, placebo-controlled
  - double blind

Study ongoing

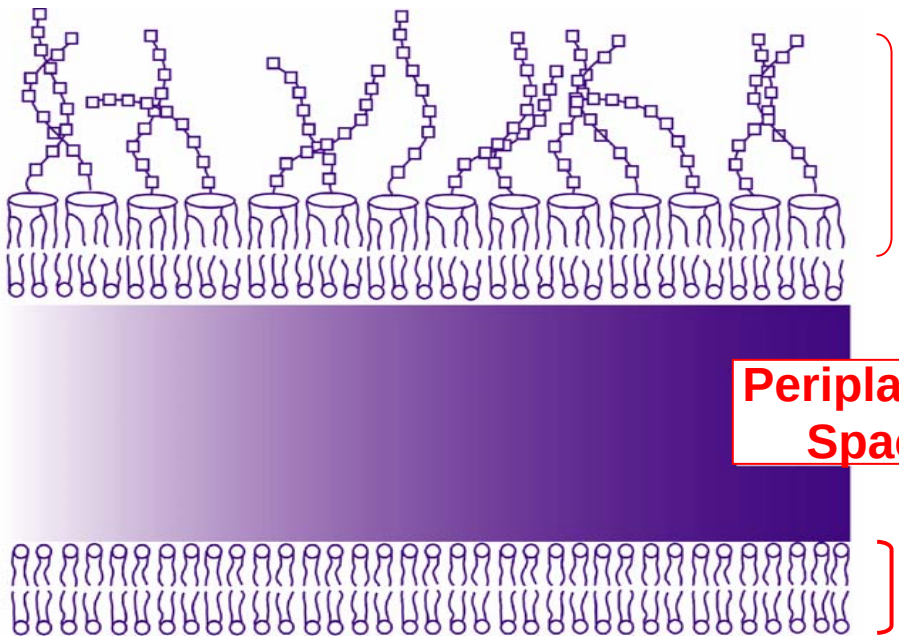
- **No serious adverse events**
- **No injection site reactions**

EluSys THERAPEUTICS, INC.

Key Features of *E. coli* that Accelerate Protein Engineering

- **High transformation efficiency for library production**
- **Fast doubling time facilitating routine manipulations**
- **Many specialized strains and tools are available for cloning and protein expression**
- **Amenable to quantitative FACS sorting (10^7 clones per hour)**

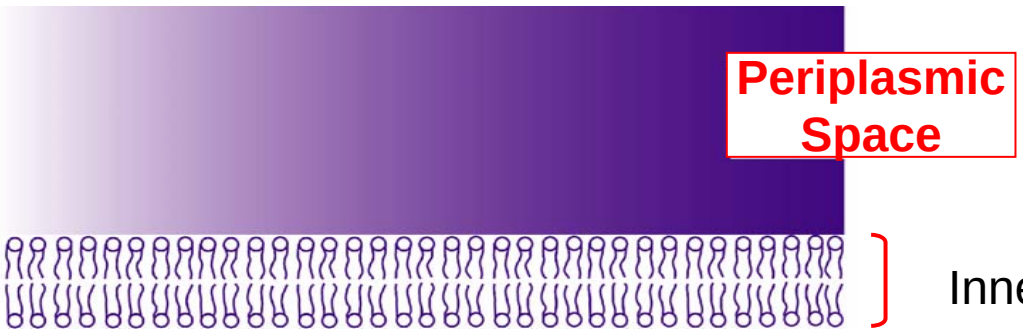
Key Features of *E. coli* that Accelerate Protein Engineering



***E. coli* cell envelope**

➢**Dual membrane structure with periplasmic space that can facilitate interactions between co-expressed proteins**

Key Features of *E. coli* that Accelerate Protein Engineering



➢**Dual membrane structure with periplasmic space that can facilitate interactions between co-expressed proteins**

➢**Outer membrane can be selectively permeabilized with detergent or largely removed by spheroplasting**

Anchored Periplasmic Expression (APEx)

- **Isolation of ligand binding proteins √√√√ (similar to yeast display)**

- **Protein:protein interactions √√√√ (quantitative)**

- **Expression maturation √√**

- ***Enzyme engineering* yes, for hydrolytic enzymes**

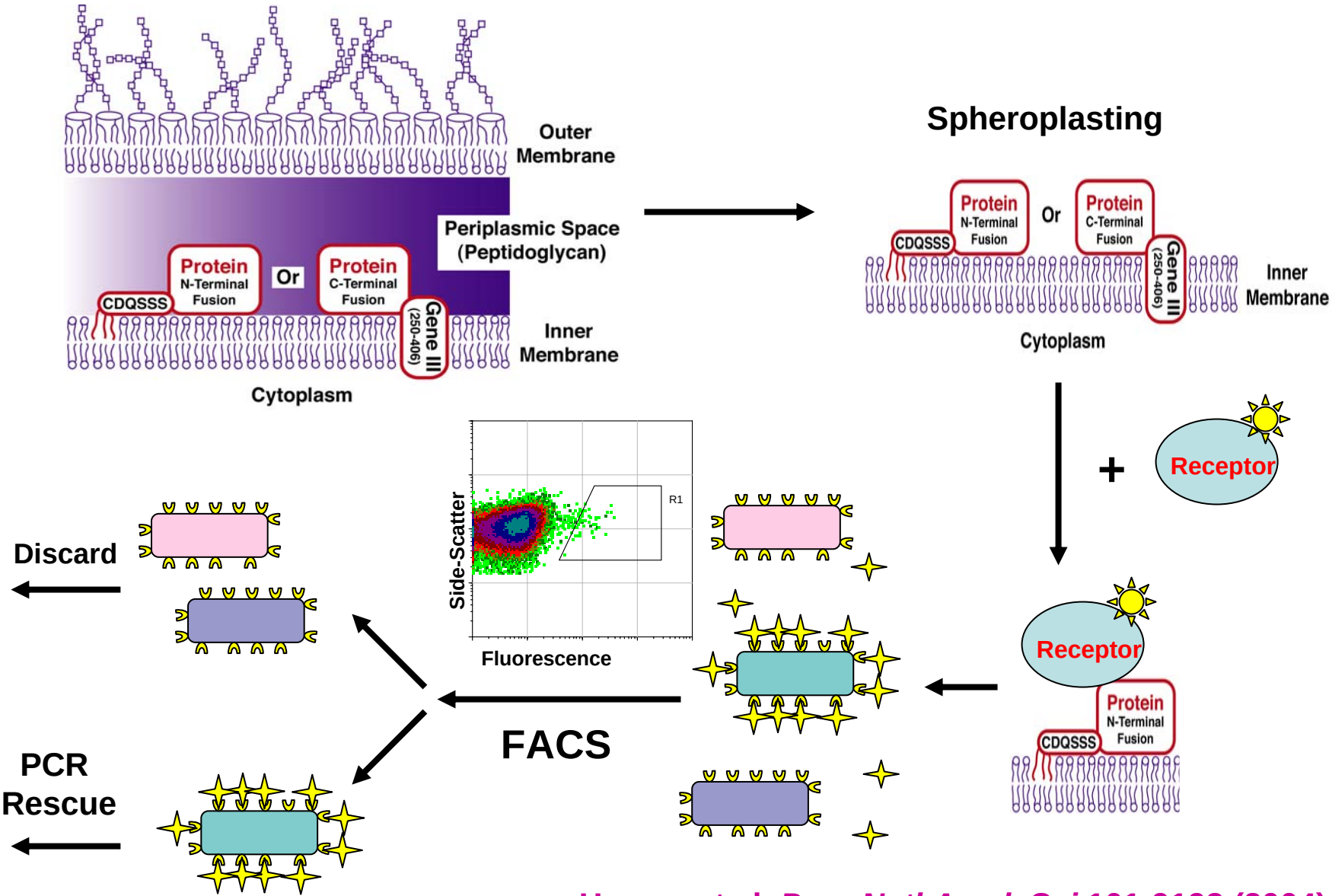
- ***Suitable for the engineering of multi-subunit proteins & proteins with complex cofactors***

- ***Membrane protein engineering***

Commercial considerations

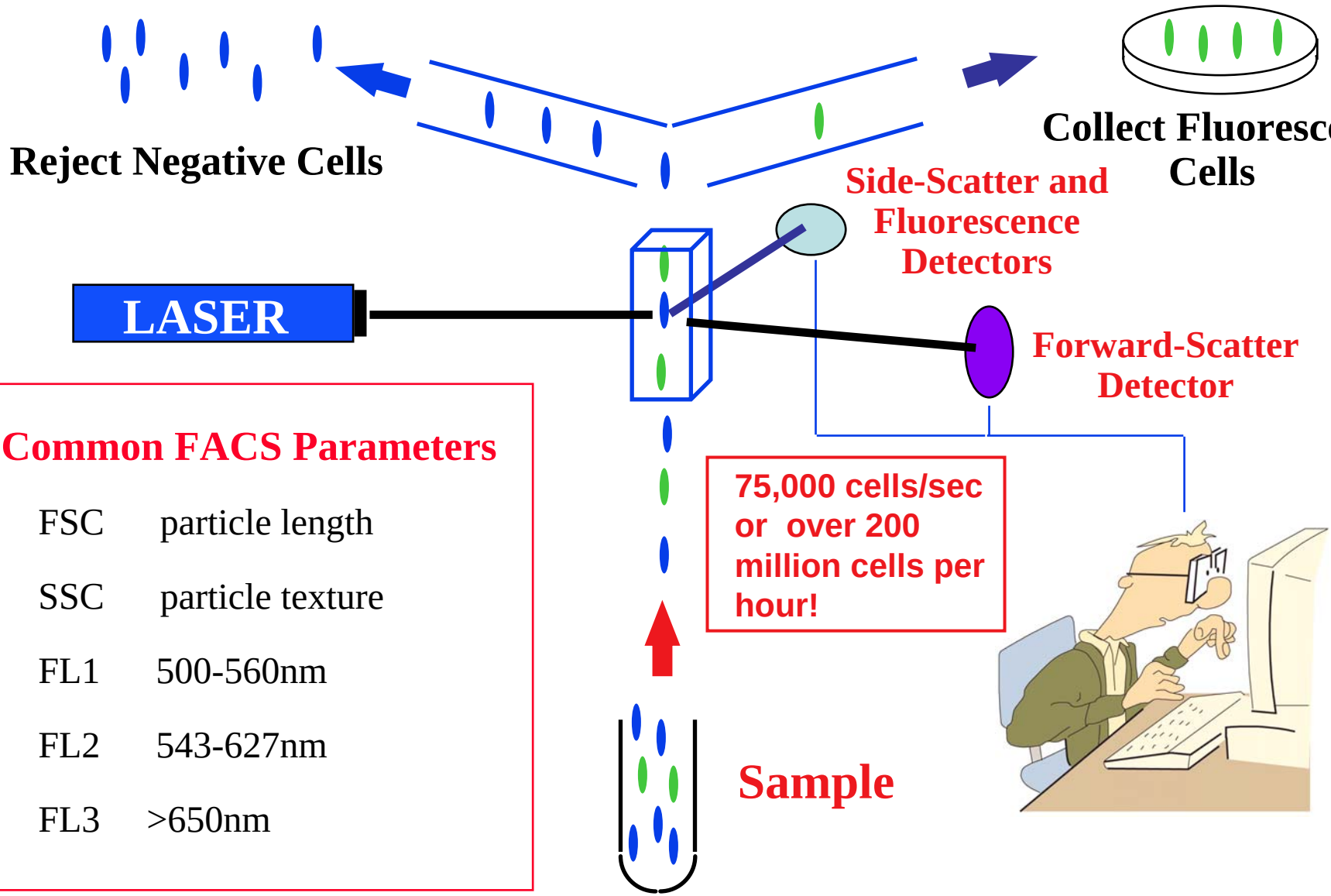
-Available; licensed by Merck, Pfizer, Lilly, and others

Anchored Periplasmic Expression (APEx)



Harvey et al. *Proc Natl Acad. Sci* 101:9193 (2004)

Fluorescent Activated Cell Sorting (FACS)

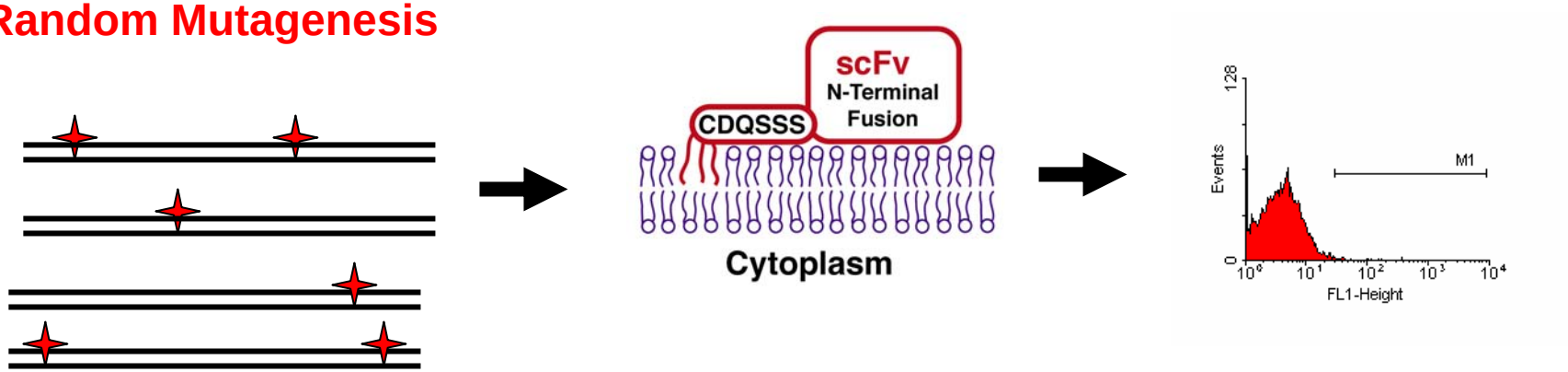


Common FACS Parameters

FSC	particle length
SSC	particle texture
FL1	500-560nm
FL2	543-627nm
FL3	>650nm

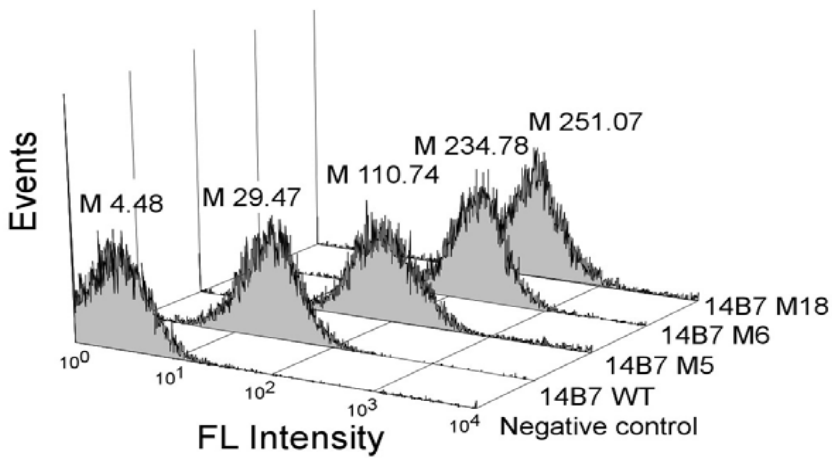
Isolation of High Affinity Anti-PA Antibodies

Random Mutagenesis



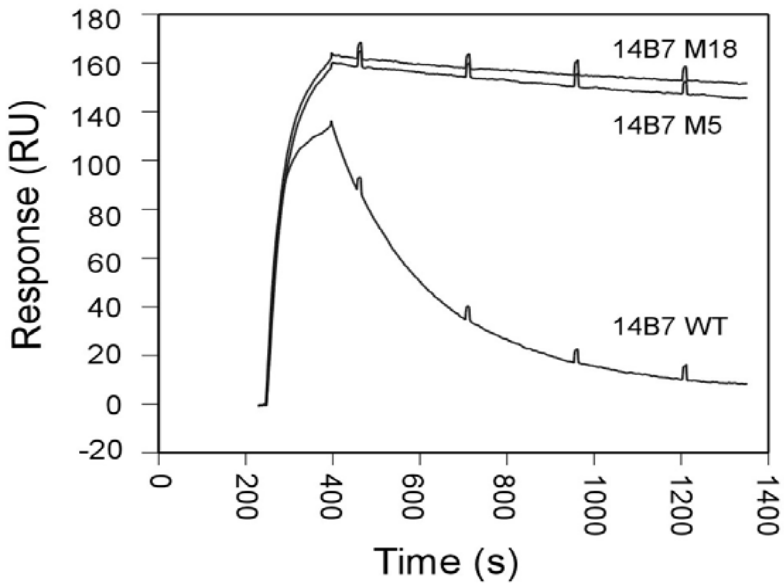
A

Flow Cytometry Scans
200nM PA-BodipyFL



B

BIACore Results



Ultra-High Affinity PA Neutralizing Antibodies

Antibody Fragment	k_{on} (*10^5 M^{-1} sec^{-1})	k_{off} (*10^{-4} sec^{-1})	K_D (nM)
14B7	7.1 ± 0.3	30 ± 0.8	4.3
1H	6.4 ± 0.8	1.7 ± 0.3	0.25
M18.1	11 ± 4	0.24 ± 0.03	0.021
M18.1 Optmized	8.3 ± 2	0.10 ± 0.02	0.012

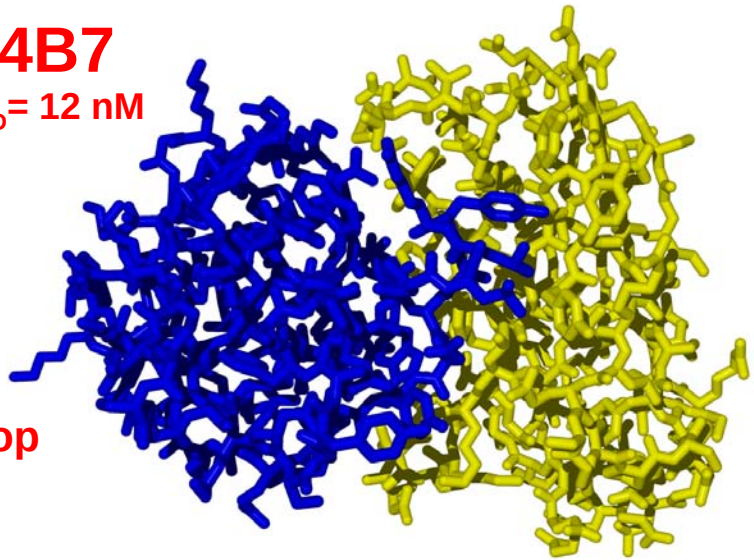
$t_{1/2}$ ~ 20 hours

US Patent 6, 916,474

X-tal Structures: Before and After APEx

14B7

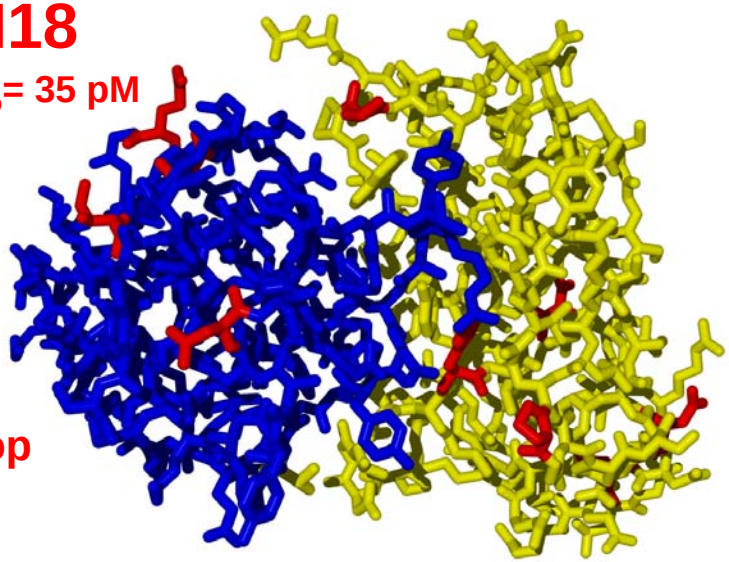
K_D= 12 nM



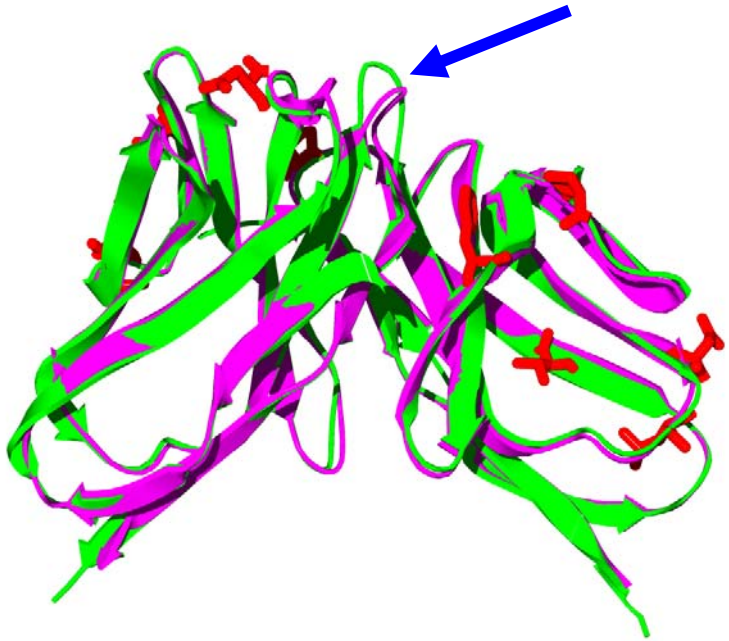
Top

M18

K_D= 35 pM



Top



Side

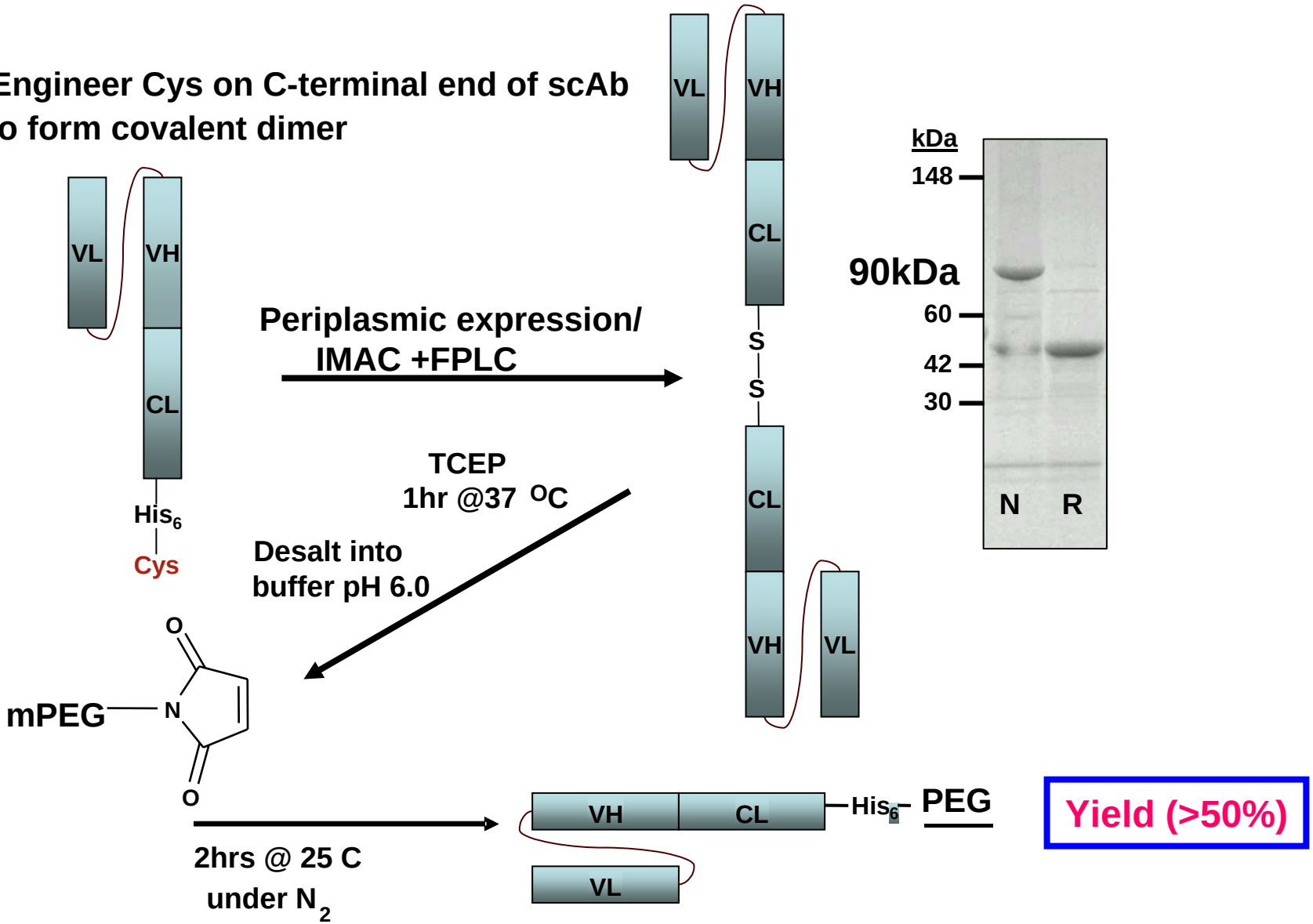
Overlay

Top

Jennifer Maynard, Clint Leyseth, Art Monzingo, Jon Robertus

Preparative ScAb PEGylation

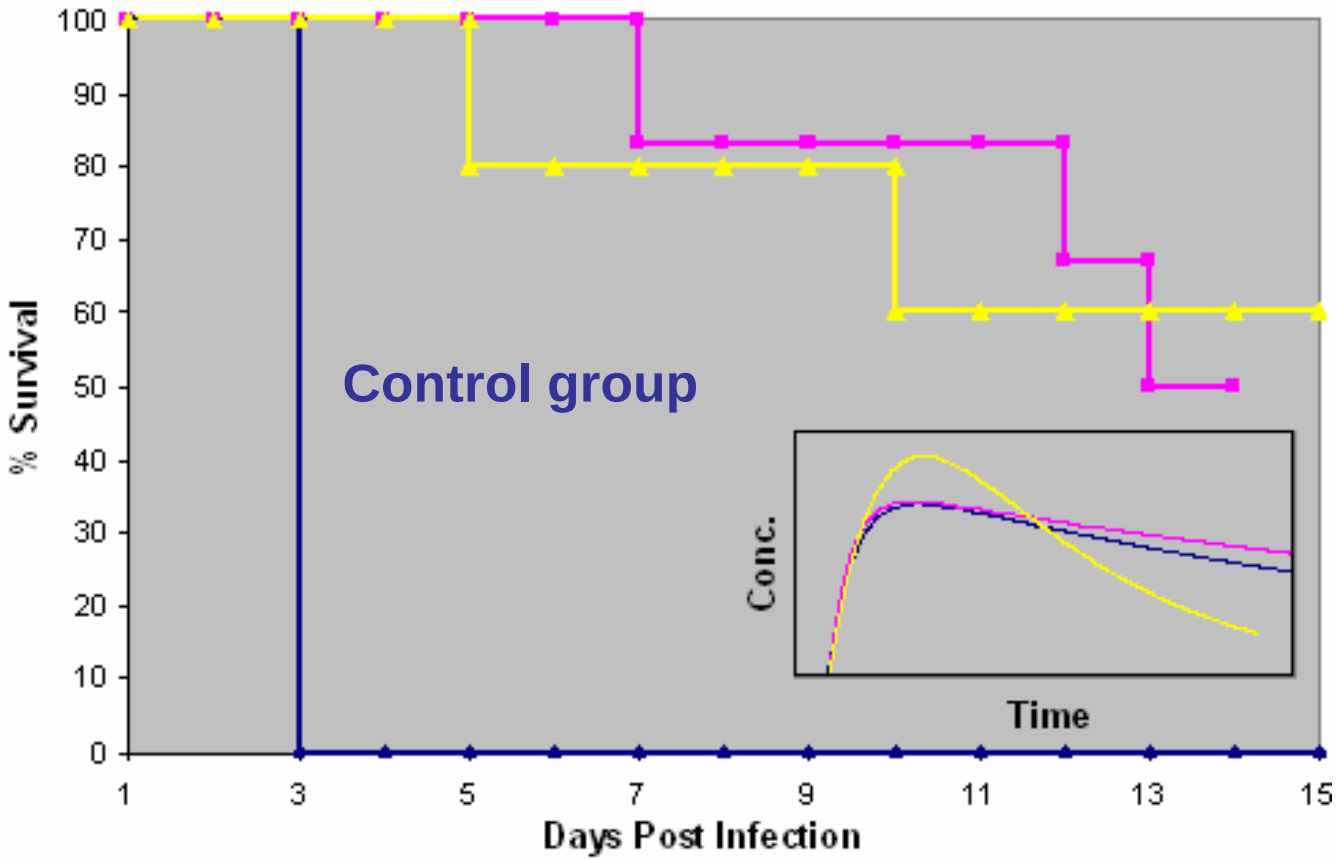
- Engineer Cys on C-terminal end of scAb to form covalent dimer



Yield (>50%)

Protection Against Challenge w/ Anthrax Spores

- Guinea pig model
- Challenge w/ 500 LD_{50} spores



Mabry *et al. Infection and Immunity 73:8362-8 (2005)*

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Passive Protection by Polyclonal Antibodies against *Bacillus anthracis* Infection in Guinea Pigs

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Microbial Pathogenesis 38 (2005) 209–225

www.elsevier.com/locate/micpath

The detection of protective antigen (PA) associated with spores of *Bacillus anthracis* and the effects of anti-PA antibodies on spore germination and macrophage interactions

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[a]*United States Army Medical Research Institute of Infectious Disease (USAMRIID), Bacteriology Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA*

[b]*USAMRIID, Diagnostic Systems Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA*

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Received 1 November 2004; received in revised form 14 February 2005; accepted 14 February 2005

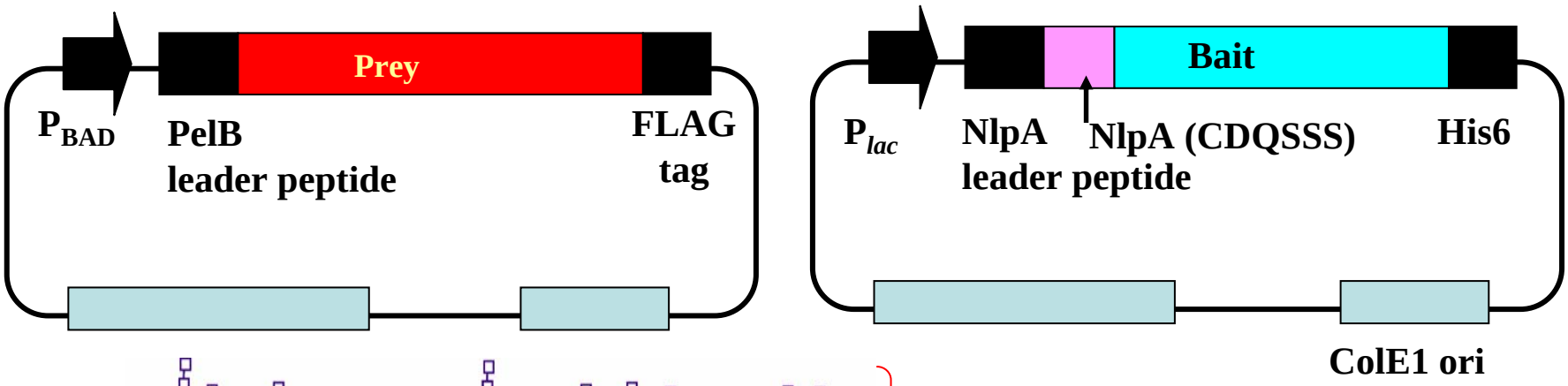
Available online 22 April 2005

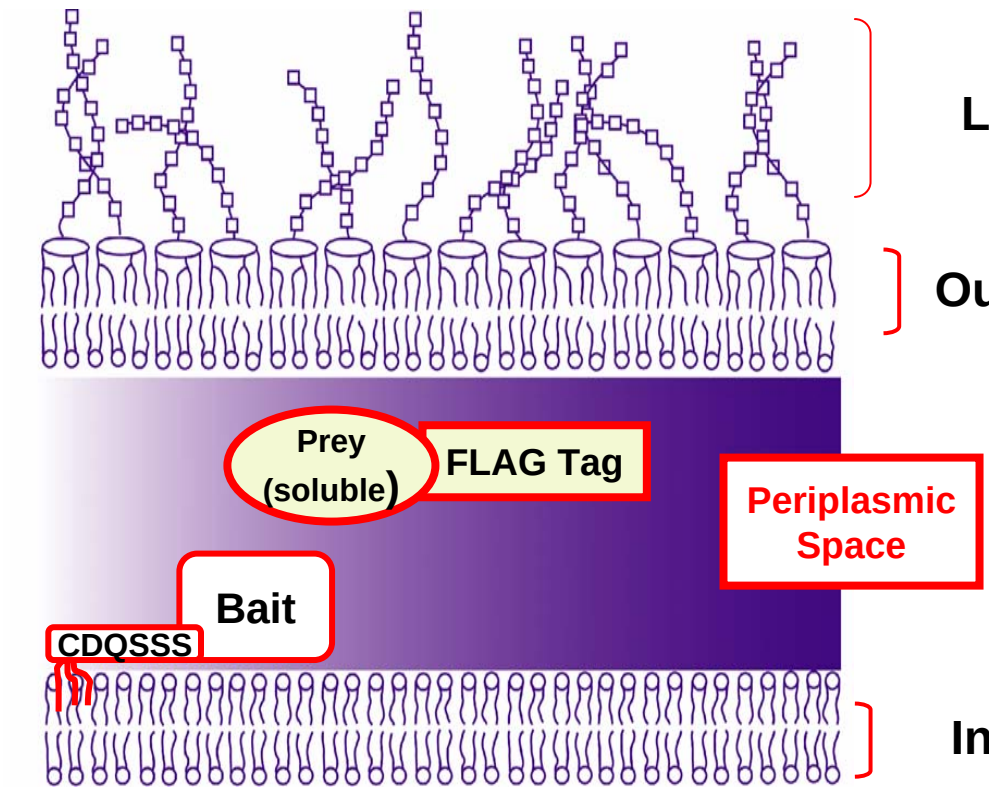
Abstract

The protective antigen (PA) component of the anthrax toxins is an essential virulence factor of *Bacillus anthracis* and is the major protective immunogen. The kinetics of PA production during growth of *B. anthracis*, and the roles of anti-PA antibody in host immunity are not clearly defined. Production of PA by the vegetative organisms peaks during the shift from exponential to stationary phase of growth. Recently, PA was also found to be associated with spores. In our study, PA-specific mRNA was detected in spores by RT-PCR within 15-min of exposure to germinant. PA protein was detected by immunomagnetic electrochemiluminescence (ECL) on spores within 1 h of exposure to a germination medium and was rapidly released into the supernatant. PA was not demonstrated on ungerminated spores by RNA analysis, ECL, or spore-based anti-PA ELISA; however, it was detected on ungerminated spores by immunoelectron microscopy (immunoem). In rabbits, PA induces polyclonal antibodies (Abs) that, in addition to their anti-toxin neutralizing activities, exhibit anti-spore activities. In this study, the anti-spore effects of a human monoclonal Ab specific for PA (AVP-hPA mAb, Avanir Pharmaceuticals) were characterized. AVP-hPA mAb retarded germination in vitro, and enhanced the phagocytic and sporicidal activities of macrophages. The activities were comparable to those of the polyclonal rabbit anti-rPA Ab. Assays to detect germination inhibitory activity (GIA) in serum from vaccinated mice and guinea pigs suggested a possible role for anti-PA Abs in protection. Thus, anti-PA Ab-mediated, anti-spore activities may play a role in protection during the early stages of an anthrax infection.

Keywords: Anthrax; *Bacillus anthracis*; Spores; Protective antigen; Anti-PA antibodies; Immunity

II. APEx 2-Hybrid Technology
(A quantitative high throughput approach)





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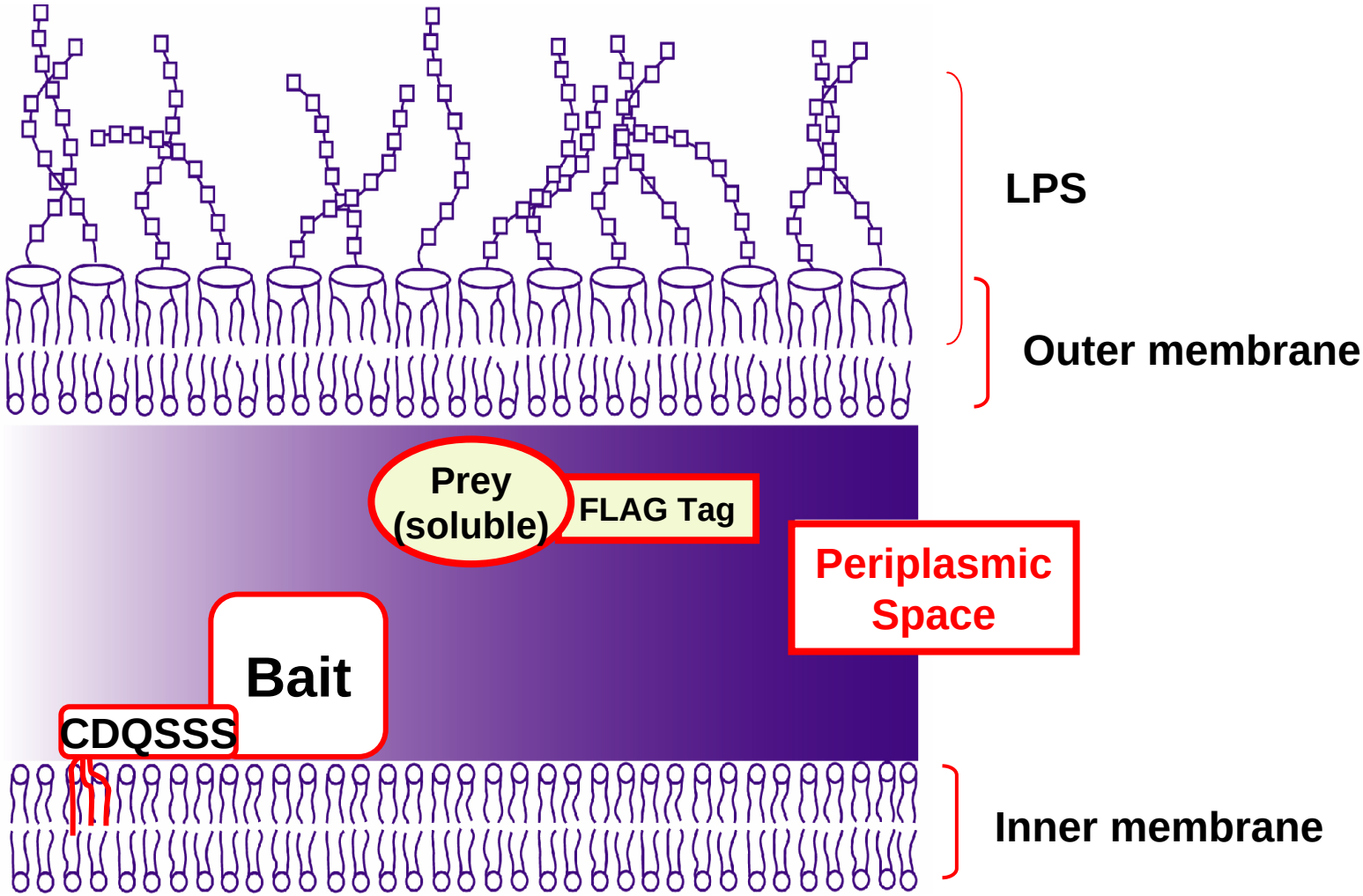

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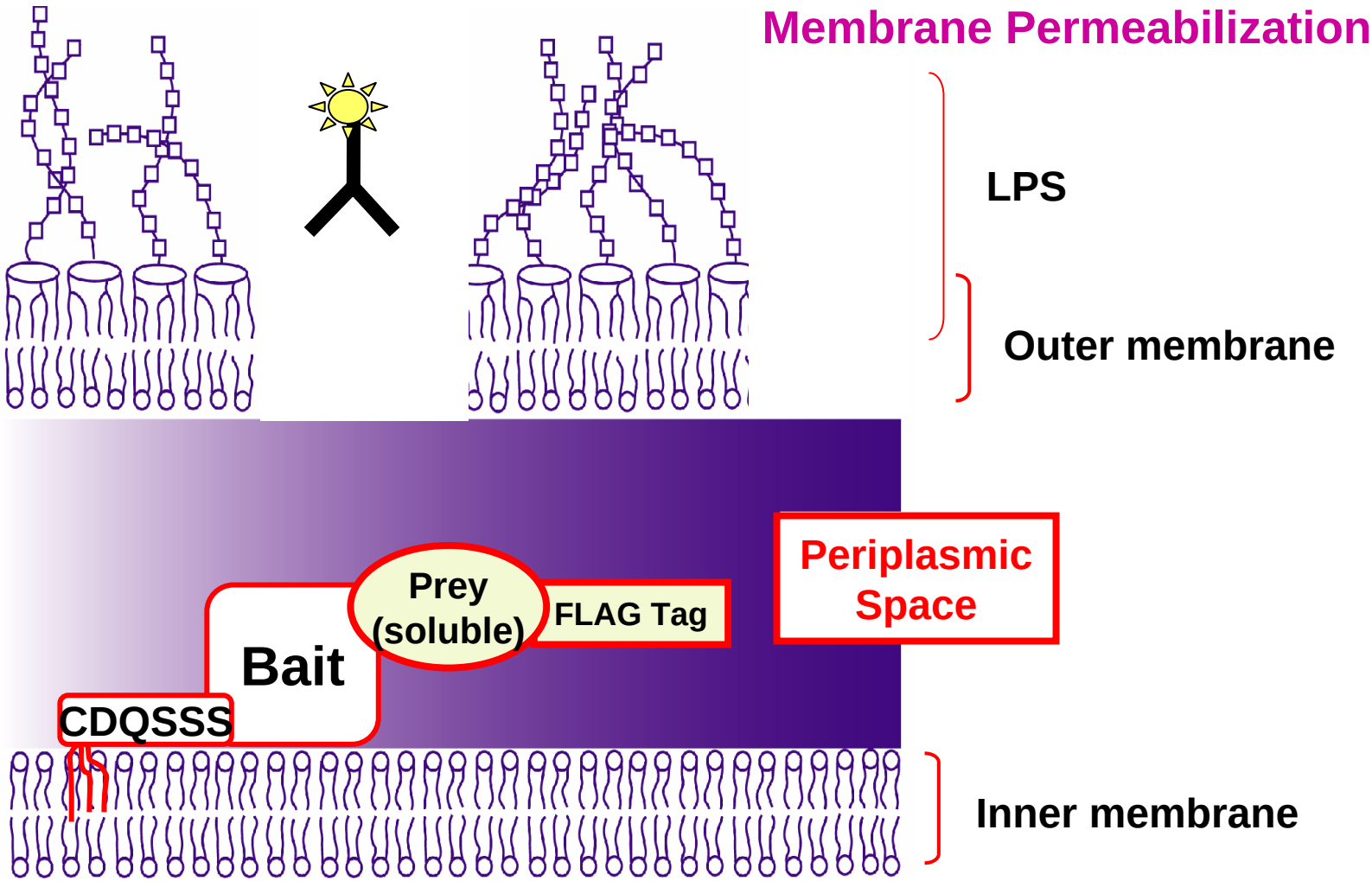

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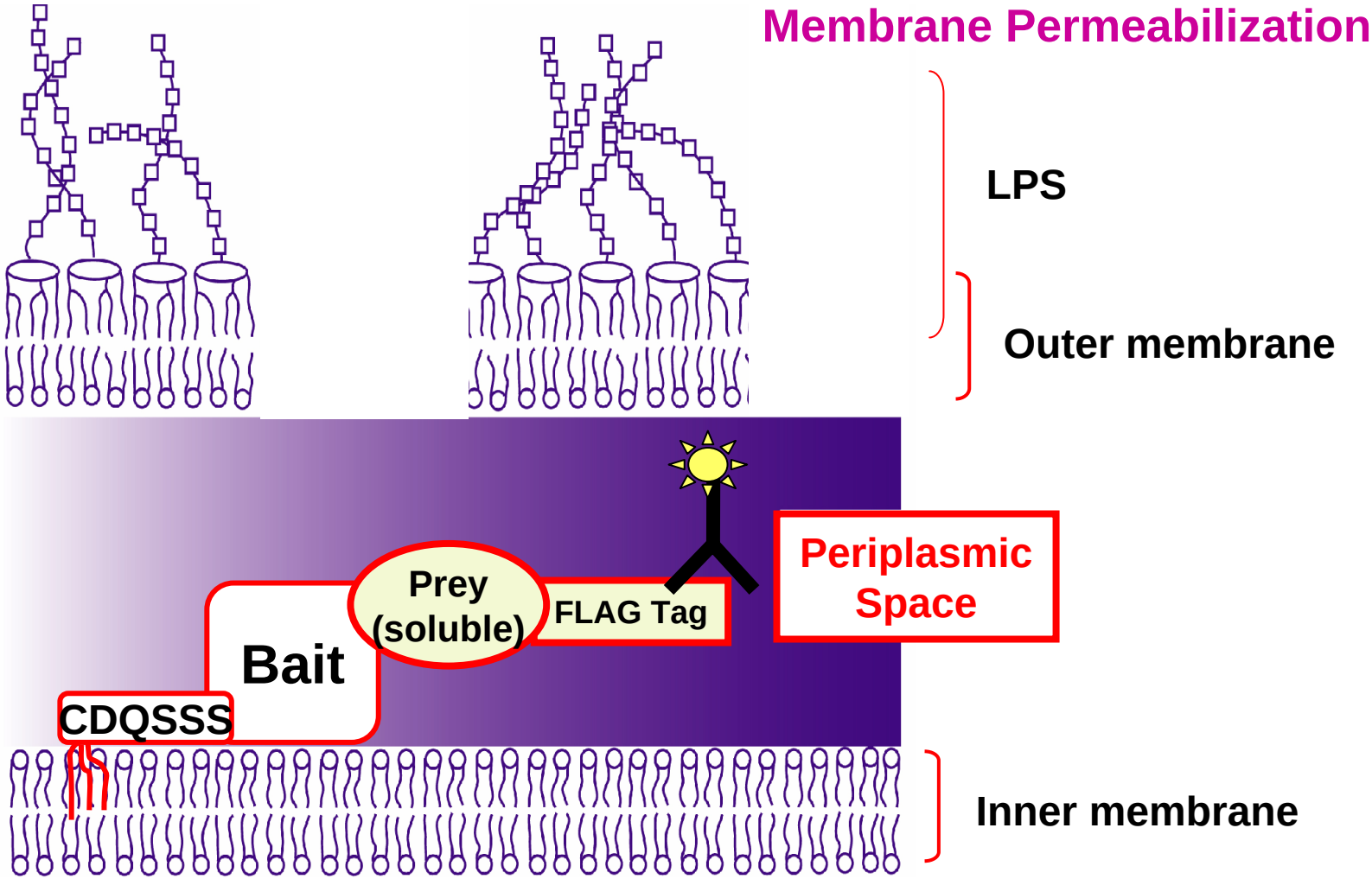
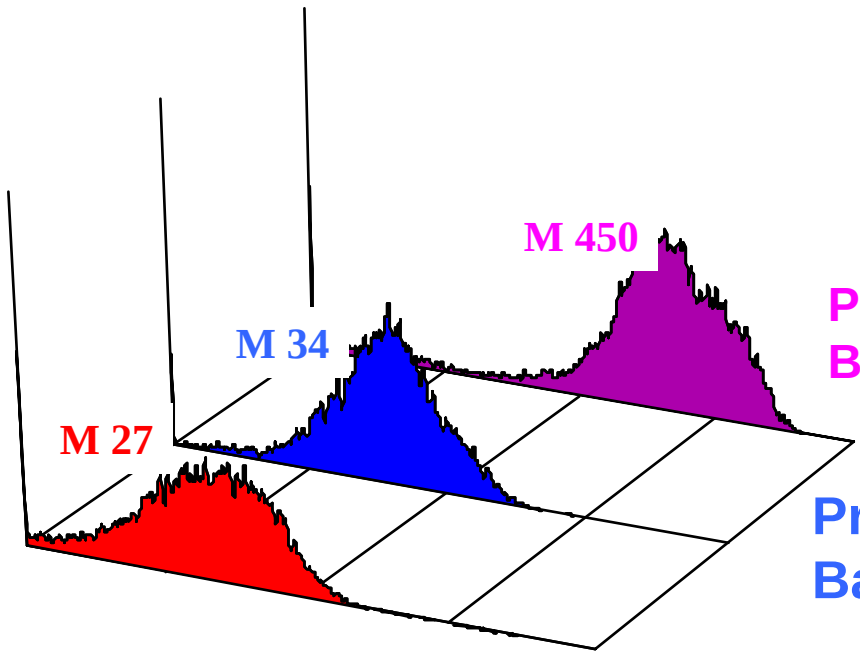


Cell fluorescence proportional to Bait:Prey dissociation rate

Detecting Protein:Protein Interactions by APEx 2-Hybrid



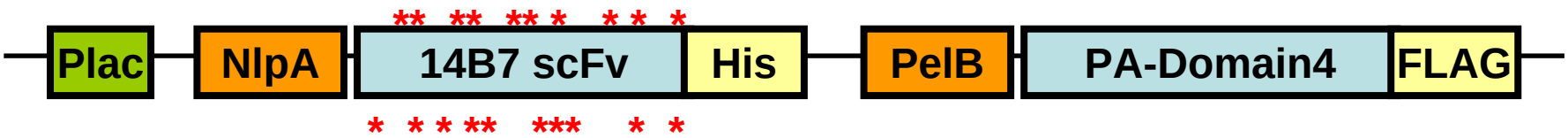
Prey: Antigen (PA-D4)
Bait: anti-PA scFv

Prey: Antigen (PA-D4)
Bait: unrelated scFv (26-10 anti-dig)

Prey alone: Antigen (PA-D4)

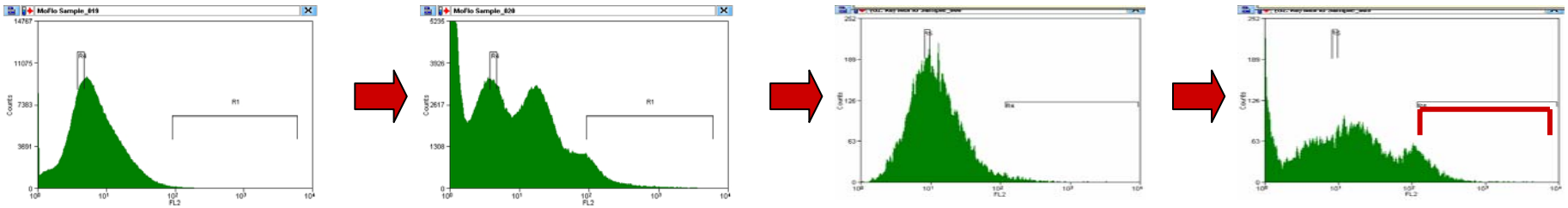
Isolation of High Affinity Antibodies to Endogenously Expressed Antigen

Dicistronic Expression of Ab &Ag Genes in Single Plasmid)



Random mutagenesis by error-prone PCR

Sorting of positive clones by FACS on a Cytomation MoFlo



Library 1st round sorting

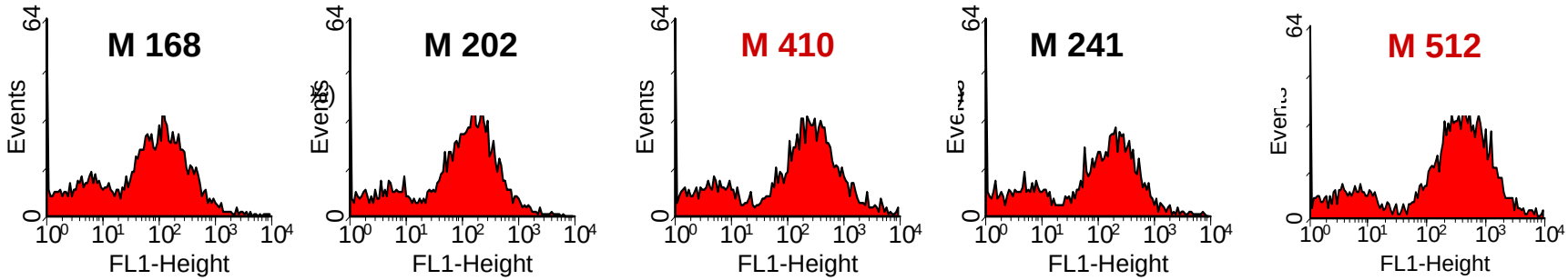
1st round Re-sorting

2nd round sorting

2nd round Re-sorting

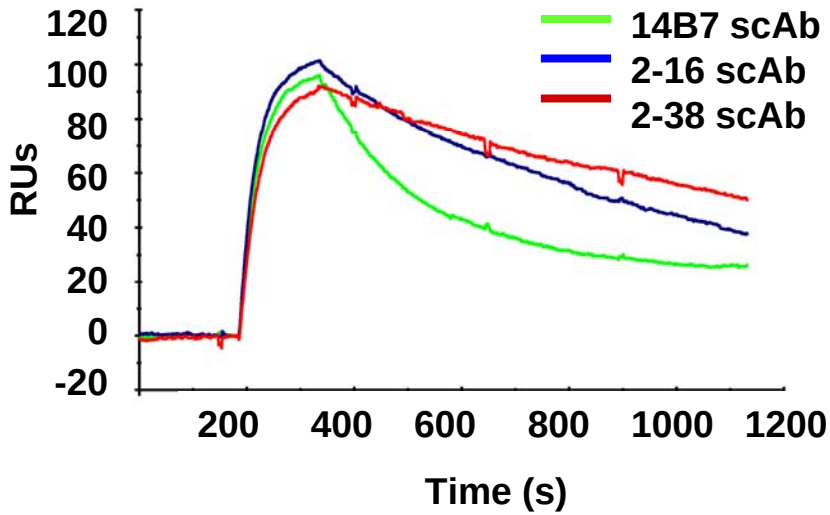
Analyzed individual clones from this round for improved affinity

Individual scanning after 2nd round sorting



14B7 | Clone # 2-4 | Clone # 2-16 | Clone # 2-30 | Clone # 2-38

SPR analysis of anti-PA scAb binding to PA



scAb	K_D (pM)	K_{off} (sec^{-1})
14B7	4600	2.8×10^{-3}
2-16	480	4.9×10^{-4}
2-38	270	2.6×10^{-4}

>17 fold increase in affinity after one round of mutagenesis

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